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A STUDY OF AN ORGANISM FROM NEPHRITIS IN SHEEP

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In 1918 Fitch, Boyd and Billings[1] reported on the isolation of an organism from ovine nephritis. Since then we have isolated a similar organism from two other cases and have made a study of its more important characteristics.

Ovine nephritis is not commonly reported in the literature. According to Leblanc acute nephritis is almost unknown among sheep. Baker[2] does not mention it, nor does Oppermann.[3]

The three cases from which we have isolated the organism here described occurred in rams. The symptoms in each case were obscure, and a diagnosis of the real cause of the trouble was not made until necropsy.

The necropsy findings in the first case were: "The kidneys showed active inflammation; the capsule was adherent and when removed particles of pus were discharged from small abscesses located in the cortical region. In the pelvis there were several cavities (1-2 cm.) which were filled with pus. The ureters were enlarged, hyperemic, and edematous and contained some pus. The musculature of the bladder was not involved, but the mucous surface was inflamed and studded with hemorrhages of punctiform variety. Microscopic examination of properly stained sections showed the typical changes of a purulent nephritis."

The cultural findings as reported in this first article were as follows: "Cultures made on nutrient agar from the kidneys and incubated at 37 C. showed sparse growth. After three days' incubation a few small grayish white colonies appeared on each of the cultures. Microscopic examination showed a short (1-2μ) rod-shaped and non-motile organism, with rounded ends, existing usually singly, sometimes in pairs and short chains. It stains well with carbol fuchsin and methylene blue. It is gram-negative. Grows quite vigorously in infusion bouillon with the production of considerably rather viscid sediment. This growth appears at first in the top layers of the bouillon and then gradually settles. It has no action on milk. On agar the organism grows very sparingly with the production of small grayish white colonies. The growth in

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[1] Cornell Veterinarian, 1918, 8, p. 241.

[2] Diseases of Sheep, 1920.

[3] Lehrbuch der Krankheiten des Schafes, 1919.

bouillon containing glucose, lactose and sacchrose is essentially the same as described in plain bouillon. No gas is produced in any of these sugars nor is the reaction changed to litmus."

This organism died out, and we did not have another case until January, 1920. This sheep was sick 48 hours. Digestive disturbances were suspected, but on necropsy an acute nephritis and an inflammation of the mucous membranes of the bladder and ureter were found. Cultures were made on slant agar from the kidneys and bladder. From the kidneys an organism grew in pure culture. From the mucous surface of the bladder the cultures were mixed, some containing an organism of the B. coli type and many colonies of the same organism found in the kidneys. This organism is designated as strain B.

In June, 1920, an Oxford ram was found dead in the pasture. The animal had been noted as not doing well for 2 weeks previously and stomach worms were suspected as the cause. Necropsy showed that the kidneys were enlarged about twice. The ureters were enlarged and the mucous membrane highly congested. The mucous surface of the wall of the bladder was congested and in places hemorrhagic. The kidneys showed ecchymotic areas (0.5 to 1 cm.) on the surface beneath the capsule. The capsule was slightly adherent. Cultures from the cortex of the kidney on plane agar gave very slight growth. Cultures prepared from the medulla and pelvis on incubation showed many colonies of B. coli-communis. Cultures prepared on serum agar from the cortex showed many small white colonies which on further study were shown to be closely allied to the strain mentioned previously, and hereafter known as strain F.

Morphology.—The organism is a short 1-2μ rod with rounded ends. Occasional coccoid forms are seen, especially in old agar slant cultures. Sometimes long involution types are noted from agar cultures. It is nonmotile, and no spores or granules are visible. It stains readily with the aniline dyes. It does not, however, take Gram stain.

Cultural Characteristics.—In meat infusion broth, especially in early generations, growth is sparing, becoming visible in 48 hours. In later generations growth is moderate in 24 hours. Cloudiness is uniform with a developing semiviscid white sediment which also adheres to the sides of the tube and forms a viscid terminal ring. In broth containing certain of the carbohydrates, a thin surface pellicle is formed. In cultures incubated for 5 days, the sediment becomes quite heavy. Indol is not produced in Dunham's peptone and growth is very sparing.

On beef infusion agar, especially when first isolated, growth is very scant. On agar containing 10 per cent. horse serum growth is much more vigorous. The colonies are discrete, grayish white, translucent and show a bluish iridescence. After 4 generations of artificial cultivation, the growth becomes more confluent and filiform. The growth shown by the F strain after long continued cultivation tends to become heavier than that of the B strain.

On gelatin the strains will not grow at 20 C. Growth at 37 C. was slight and no liquefaction was produced. Growth in incubated gelatin was restricted to a few colonies on the surface.

Small white colonies are produced on Loeffler's serum; no liquefaction takes place.

No change is noted in milk after 7 days' incubation. Litmus milk likewise shows no change in reaction.

Strains B and F were streaked on defibrinated blood agar. One c c of blood was used for 10 c c of agar. No hemolysis was noted after 48 hours' incubation at 37 C.

Fermentation Studies.—A careful study was made of the change in the hydrogen-ion concentration in sugar-free beef infusion broth containing various carbohydrates. The method followed was the one described by Medalia[4] in determining the P_H values of culture mediums. We did not follow the method described by him to determine the effect of organisms on indicators. The indicator studies were checked from time to time by the potentiometer. The various carbohydrate mediums were sterilized by filtration as described by Fitch and Billings.[5] The details of this work are contained in table 1.

TABLE 1

AVERAGE PH VALUES OF EACH STRAIN IN DUPLICATE IN VARIOUS CARBOHYDRATES *

Strain	Strain B		Strain F		Uninoculated Controls
Arabinose	6.8	7.0	6.2	6.3	6.8
Xylose	6.6	6.7	6.4	6.3	6.8
Rhamnose	7.2	7.3	7.2	7.2	7.2
Dextrose	7.5	7.4	6.5	6.5	7.0
Levulose	7.3	7.3	6.0	6.0	7.0
Galactose	7.4	7.3	6.3	6.3	7.1
Sucrose	7.2	7.4	7.1	7.1	7.0
Maltose	6.9	7.1	6.1	6.2	7.0
Lactose	7.2	7.2	7.0	7.0	7.0
Raffinose	7.5	7.6	7.3	7.3	7.1
Inulin	7.6	7.6	7.1	7.1	7.0
Salicin	7.5	7.5	7.2	7.3	7.0
Mannitol	7.1	7.2	6.7	6.7	7.0
Dulcitol	7.4	7.5	7.3	7.3	7.2

* The original tables giving the P_H value for each titration can be secured from the authors.

The carbohydrates were inoculated in duplicate with strains B and F, then incubated at 37 C. and the reactions determined on the 1st, 3rd, 5th, 10th, 20th and 30th day of incubation. Readings were made in terms of H-ion concentration. In order that space might be conserved we have condensed the original tables into one. The P_H values appearing in this table were compiled by adding together the 6 P_H values obtained on each day (1st, 3rd, 5th, etc.) and recorded for the strain in the various carbohydrates. Then an average titration figure was obtained from the sum of the 6.

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[4] J. Bacteriology, 1920, 5, p. 441.

[5] Ibid., p. 469.

Summarizing the results, we find that in arabinose and xylose strain B practically does not change the P_H value with a possible slight production of acid in xylose, while strain F produces a slightly acid reaction in both. Neither strain changes the reaction of rhamnose. In the hexoses the F strain produces acid while the B strain produces alkalinity. Sucrose is unchanged by both strains until the 30th day when a decided alkalinity is produced by both strains, so as to raise the average P_H value slightly above the controls. In maltose, strain B produces no change, while F produces a marked acid reaction. In lactose both strains remain practically unaltered, strain B having a slightly higher P_H value.

Raffinose of the trisaccharid group is rendered alkaline by both strains. Inulin of the polysaccharids is rendered alkaline and is more pronounced by the B strain.

Salicin of the glucoside group is rendered slightly alkaline more pronounced by the B strain.

Of the polyhydric alcohols, mannitol is rendered alkaline by the B strain while the F strain produces a slight acidity. In dulcitol a slight alkalinity is noticed more pronounced by the B strain.

Gas is not produced in any of the carbohydrate broths. The inverted vial method was used.

Several interesting observations were made in this study. We found that certain of the carbohydrate mediums in tubes with paraffined plugs had a different P_H value than in similar tubes with unparaffined plugs. This difference was noted in both inoculated and uninoculated tubes after 10, 20 and 30 day incubations. This difference apparently was not due alone to the effect of the organism but was in part caused by the continued incubation. A careful study of this feature is being planned.

Inoculation and Immunologic Experiments. It was stated in the first report on this organism that: "Subcutaneous inoculations into rabbits and guinea-pigs are yet (5 weeks) attended with no results." We have repeatedly given intravenous, intraperitoneal and subcutaneous inoculations of various quantites of fresh cultures of this organism into rabbits and guinea-pigs without apparent bad effect. The inoculated animals have remained well for two months, after which they have been killed and careful necropsy examinations have been made. No lesions have been found.

We injected one ram (1) intravenously with 2 c c of the F strain. This material was secured by washing off the growth from two 24-hour serum-agar cultures with 5c c salt solution. These cultures were the 4th generation of this strain after original isolation. At the same date we injected a second ram (2) intraperitoneally with 4 c c of a broth suspension from 2 slant agar cultures of the B strain. These injections were made at 10 a. m. The temperature of sheep 1 when injected was 102.5, at 12:15 p. m., 105, at 6 p. m. 105.8. Animal in visible distress. Dyspnea, bloody diarrhea, and bloody mucus from rectum. Died early the next morning. A careful necropsy examination was performed, the principal changes being congestion of the various internal organs, serosanguineous fluid in the thoracic cavity, and marked congestion and hemorrhages of the mucosa of the intestinal tract. Clots of blood were found in the colon. The kidneys were markedly congested. Cultures from the lungs showed the same organism as strain F. Cultures from the kidneys remained sterile. Portions of kidneys taken for microscopic examination showed parenchymatous degeneration, congestion and hemorrhage.

The temperature of ram 2 continued normal, and no evidence of disease was noted. This animal was killed some weeks later. No macroscopic evidence of disease was found. Sections of the kidneys on microscopic examination showed no evidence of change.

Ram 3 was injected subcutaneously with 2 c c of salt solution suspension of F strain prepared in the same manner as the foregoing. On the 5th, 6th and 7th days following injection, a rise of temperature to 104.6 was noted. This, however, fell to normal on the 8th day. This animal became emaciated and weak and died after 30 days, and the necropsy examination showed pleuritic adhesions, serosanguineous fluid in the thoracic cavity, pneumonia and consolidation of the anterior lobes of the lungs. One specimen of Dictyocaulus filaria was found in a consolidated portion. A few Hemonchus contortus were present in the abomasum. The kidneys were apparently normal. Cultures on horse serum agar from the various organs, incubated at 37 C. did not yield strain F from any of the organs. Microscopic sections of the kidneys showed parenchymatous degeneration.

Ram 4 was injected intravenously with 0.5 c c of suspension of F strain. On the afternoon of the same day the temperature rose to 105.2 F. The following day it was back to normal and remained so until the 5th, 6th and 7th days, when it rose to 104.2. On the 8th day the temperature again became normal and remained so. No apparent evidence of disease was noted. This animal was killed 10 weeks later and all organs appeared normal, microscopic sections of the kidneys showing no pathologic changes. Cultures from the various organs remained sterile.

Ram 5 was injected intravenously with 2 c c suspension of F strain. The temperature on following day rose to 106 F. The animal was dull. The temperature returned to normal on the next day. On 7th, 8th and 9th days, it rose again to 104.3. During this period the animal was off feed. Normal temperatures were observed thereafter. This animal was killed about 10 weeks later and appeared macroscopically normal. Cultures on serum agar from the various organs remained sterile. Microscopic sections of the kidneys showed congestion and evidence of chronic nephritis with leukocytic infiltration, especially in the cortex.

Ram 6 was injected intravenously with 2 c c suspension of B strain. A rise of temperature to 104.3 F. was noted on the 5th day. This animal was off feed for 9 days. No further evidence of disease was noted, and the animal was killed 10 week later. A subcutaneous abscess 2.5 c. m. in diameter was found at point of injection. No other macroscopic lesions were noted. Cultures made on serum agar from the kidneys, testicles and subcutaneous abscess remained sterile. Those from the lungs, liver and spleen showed colon type organisms. Microscopic examination of pus from the subcutaneous abscess showed gram-negative short rods. Sections of the kidneys showed microscopically the same changes as noted in ram 5.

An effort was made to inject into the urethra of ewe lamb 7 a suspension of the F strain. We were unable to make the injection, directly into the urethal orifice and the suspension was placed in the vagina at the urethal opening. The temperature of this animal remained normal for 5 days followng the inoculation. About 6 weeks later the animal did not eat well, had lost flesh, and was growing very weak. Fourteen days later the animal could not stand, and was killed. Both kidneys showed marked evidence of chronic nephritis. The capsules were adherent and the cortex gray in color. A very few Hemonchus contortus were found in the abomasum. Other organs were apparently normal. Cultures were made on serum agar from the various organs, and on incubation remained sterile. Microscopic sections of the kidneys showed marked parenchymatous degeneration, especially in the cortex. The glomeruli were also involved.

Blood was drawn from sheep 5, 6 and 7. The serums from these animals agglutinated strains F up to and including a 1:1000 dilution.

Strain B was not agglutinated at all. Standard technic was used.

In interpreting these results, it must be kept in mind that sheep 5 and 7 were inoculated with strain F, while sheep 6 was inoculated with strain B. It is difficult to understand the reaction obtained.

Rabbits were immunized to strains B and F, respectively. The injections were given as follows: 1 c c salt solution suspension, intravenously Sept. 25; 3 c c salt suspension, subcutaneously Oct. 2; 5 c c salt suspension, subcutaneously Oct. 12. Animals bled Oct. 20.

Serum from rabbit immunized with strain F agglutinated strain F completely up to 1 : 200 and slightly in the 1 : 500 and 1 : 1000 dilution. Strain B was agglutinated in the 1 : 20 dilution and slightly in the 1 : 50.

Serum from rabbit immunized to strain B agglutinated strain B up to 1 : 200, while strain F was negative throughout. These same rabbits were reinjected as follows; 3 c c suspension intravenously Oct. 28; 5 c c suspension intravenously Nov. 8; 5 c c suspension intravenously Nov. 20.

The rabbits were bled Dec. 27. The serum reactions were: Serum from rabbit immunized to strain F, agglutinated strain F up to 1:1000, while with strain B it gave a partial reaction in all tubes up to 1:200. Serum from rabbit immunized to strain B agglutinated both strains up to and including 1:1000.

DISCUSSION

A consideration of the foregoing data shows many things which require further study. The 2 strains from nephritis in sheep have certain cultural differences as shown in their effects on the various carbohydrates. However, it is hardly justified to separate them into different species on the basis of such minor variations. Another factor which may influence the variations, is the effect which incubation has on carbohydrate mediums as denoted by changed hydrogen-ion concentration. With phenolphthalein as an indicator of change in the reaction of the various carbohydrate mediums inoculated with the organisms, the variations in the two strains as denoted by the P_H values were not observed.

The inoculation experiments indicate that it is not easy to reproduce nephritis in sheep and recover the organism. . The experience with sheep 7 indicates that the infection may be an ascending one through the bladder and ureters. Further work on this point is under way.

The immunologic relationship of the 2 strains are peculiar. However, they are no greater than those reported in the same species by Meyer and Shaw[5a] in their immunologic studies with B. abortus and B. melitensis.

It is interesting to compare the biologic and pathogenic characters of the organism here described with those of an organism producing nephritis of horses and joint ill of foals. This organism was originally described by Theiler and Meyer[6] in South Africa in 1908. Recently this same organism has been studied by M'Fadyean and Edwards[7] in England and Magnusson in Sweden.[8] In many respects, morphologically there is a strong resemblance between this organism known as Bacillus nephritidis (Meyer), or Bacterium viscosumequi (Magnusson) and those isolated by us from sheep. Culturally there are variations, such as marked viscosity of growth by the equine organism, which is not shown to such an extent by the sheep strains. Pathogenically the equine organism seems to be specific for horses and the one here described, for sheep. Nephritis is produced by both. We have endeavored to obtain from M'Fadyean a strain of Bacillus nephritidis but he states that it is short lived and his strains are just now dead. We anticipate, however, a careful comparison of these two organisms. A study of the pathologic changes in nephritis of sheep is under way, and will be presented in another paper.

CONCLUSIONS

An organism for which we propose the name Bact. nephritidis var. ovis has been isolated from three cases of ovine nephritis.

Two strains of this organism have slightly different PH values in various carbohydrate mediums.

Further experiments are necessary before the channel of infection of this organism can be told with certainty.

This organism is nonpathogenic for guinea pigs and rabbits.

Apparently a somewhat close relationship exists between the organism here described and Bacillus nephritidis-equi, Meyer.

The serologic investigations do not yet permit positive conclusions.

[5a] J. Infect. Dis., 1920, 27, p. 173.

[6] Transvaal Dept. Agriculture, Report of Government Veterinary Bacteriologist, 1908-9.

[7] Jour. Comp. Path. and Therap., 1919, 32, p. 42.

[8] Ibid., p. 143.